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REMARKS

After amending the claims as set forth above, claims 79, 81-85, 87-90 and 93-95 will be pending in this application. All other claims have been cancelled.

Claim 83, which is the sole independent claim in the case, is directed to plants that contain plant cells that express a heavy chain single polypeptide product which is derived from an antigen-specific immunoglobulin comprising a heavy and light chain. According to the method, the plant cells contain nucleic acid encoding the immunoglobulin heavy chain polypeptide a leader sequence which forms a secretion signal for the single polypeptide product. As discovered by the inventors, proper processing of the heavy chain polypeptide product is required so that the heavy chain can form an antigen-specific immunoglobulin in the plant cell when the cell also expresses the light chain. The current amendments make clear that the plant cells do not contain nucleic acid encoding the light chain. Thus, claim 83 covers transgenic plants which separately express the heavy chain of an antigen-specific immunoglobulin. As described in the instant application, such plants can be crossed with, for example, a light chain expressing plant to yield offspring which express heavy and light chains that are processed and assembled into antigen specific immunoglobulin.

Claims 79 and 81-83 have been amended herein. The amendments are supported by the specification and raise no issue of new matter.

The specification has been amended by deleting reference to government support, added previously to page 1, line 4 of the application by preliminary amendment. Applicant has determined that the previous amendment was in error and requests deletion. This amendment raises no issue of new matter.

REQUEST TO CORRECT INVENTORSHIP

A request to correct inventorship under 37 C.F.R. § 1.48(b) was previously filed along with th Request for Continued Examination. However, the Office Action of

January 2, 2003, does not indicate whether or not the request was granted or even acted upon. Applicant requests that the examiner indicate in the next action whether inventorship will be changed in accordance with the request. If the request has been lost, the examiner is urged to contact the undersigned for a replacement copy.

CLAIM OBJECTIONS

Claim 79 is objected to because it refers to claim 43, which has been withdrawn from consideration by the examiner. Applicant has similarly amended claims 81 and 82.

REJECTION UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

The rejection of Claim 83 and 84-90 and 93-95 under 35 U.S.C. § 112, second paragraph as being indefinite in the recitation of "said nucleotide sequences" in part b) has been obviated by amendment herein.

The rejection of Claim 83-90 and 93-95 under 35 U.S.C. § 112, second paragraph as being indefinite because "derived" is allegedly unclear as to how much of the heavy chain is derived, is respectfully traversed. It is noted that there is no basis given for why this term is unclear. "Derived" is not indefinite because the skilled artisan would know how to choose what portion of a heavy chain encoding nucleic acid sequence it would need under the circumstances. The claim requires that the heavy chain be capable of assembly with a light chain to produce and antigen specific immunoglobulin in a plant cell. The field of antibody engineering is highly developed and the skilled artisan would know what is needed and what could be eliminated or modified and still meet the functional requirement. Applicants' specification at page 3, lines 1-6 (emphasis added) provides support for this view.

> One of the most useful aspects of using a recombinant expression system for antibody production is the ease with which the antibody can be tailored by molecular engineering. **This allows the production of antibody fragments and singl - chain molecul s, as well as the manipulati n of full-l ngth**

> antib dies. For example, a side [sic] range of functional rec mbinant-antib dy fragments, such as Fab, F_v, single-chain and single-domain antibodies, may be generated.

This broad ranging suggestion to engineer immunoglobulins is reflective of the art as a whole. For example, U.S. Patent no. 4,816,567 to Cabilly et al., filed in 1983 describes a wide variety of antibody fragments that go well beyond those available by proteolytic processing. The Summary of the Invention of the Cabilly patent is shown with emphasis below.

> The invention relates to antibodies and to non-specific immunoglobulins (NSIs) formed by recombinant techniques using suitable host cell cultures. . . .
>
> Finally, either the light chain or heavy chain alone, **or portions thereof**, produced by recombinant techniques are included in the invention and may be mammalian or chimeric.

Cabilly also defines "altered antibodies" at col. 7, beginning at line 7. The definition states as follows with respect to the power of recombinant technology (emphasis added):

> Because of the relevance of recombinant DNA techniques to this invention, one need not be confined to the sequences of amino acids found in natural antibodies; antibodies can be redesigned to obtain desired characteristics. **The possible variations are many and range from changing of just one or a few amino acids to the complete redesign, for example, the constant region**.

Cabilly further elaborates on "altered antibodies" in the context of chimeric antibodies at col. 15, beginning at line 35 (emphasis added).

> Altered antibodies present, in essence, an extension of chimeric ones. Again the techniques of D.1 and D.2 are applicable; however, rather than splicing portions of chain(s), the **suitable amino acid alterations, deletions or additions** are made using available techniques such as mutagenesis (supra).

As shown above, the term "derived" would be understood by one skilled in the art when this term is read in light of the specification. When one skilled in the art would

und rstand all of th language in the claims when read in light of the specification, a claim is not indefinite. *Rosemount Inc. v. Beckman Instruments, Inc.*, 727 F.2d 1540, 1547, 221 USPQ 1, 7 (Fed. Cir. 1984), *Caterpillar Tractor Co. v. Berco*, S.P.A., 714 F.2d 1110, 1116, 219 USPQ 185, 188 (Fed. Cir. 1983). According, the rejection is without basis and should be withdrawn.

The rejection of Claim 84 and 85-87 under 35 U.S.C. § 112, second paragraph as being indefinite in the recitation of "is a multimer" is respectfully traversed. It is alleged that it is unclear how a single polypeptide can be a multimer. Applicant points out that it is well known that a single polypeptide may associate with another polypeptide to form a multimer. The claims require nucleotide sequence encoding an immunoglobulin single polypeptide product but do not limit association of the product once produced.

REJECTION UNDER 35 U.S.C. § 102 OVER DÜRING

The rejection of claims 81-83, 88-90 and 93 under 35 U.S.C. § 102(b) as being allegedly anticipated by the During Dissertation is respectfully traversed.

The crux of the examiner's argument is that the claims were not limited to heavy chain producing plants. The examiner alleges that the claims cover plant cells that contain nucleic acid encoding a heavy and a light chain, such as described in the During dissertation.

Although it is Applicant's position that the claims as previously constituted did not cover cells that produce a heavy and a light chain, express language to this effect has now been added to eliminate this issue from the case. Thus, claims are directed to cells that contain nucleic acid encoding an immunoglobulin heavy chain polypeptide but do not contain nucleic acid encoding the light chain. The claims do not encompass cells that express a heavy and light chain heterodimer. As such, During as a single reference fails to disclose the essential requirement of a plant cell that contains nucleic acid encoding a heavy chain but not a light chain. Anticipation over prior art under 35 U.S.C. § 102 requires the disclosure in a single prior art reference of each element of the claim under consideration. *In re Spada* F.2d, 15 USPQ2d 1655 (Fed. Cir. 1990); *In re Bond*, F.2d, 15

USPQ 1566 (Fed. Cir. 1990); *Soundscriber Corp. v. U.S.* 360 F.2d 954, 148 USPQ 298, 301, *adopted* 149 USPQ 640 (Ct. Cl.) 1966. Acc rdingly, the rejection for anticipation over During fails and should be withdrawn.

REJECTION UNDER 35 U.S.C. § 102 OVER GOODMAN

The rejection of claims 81-83, 88-90 and 93-94 under 35 U.S.C. § 102(e) as being allegedly anticipated by Goodman (U.S. No. 4,956,282) is respectfully traversed. The examiner indicates that this rejection is applied for the same reasons as for During. Thus, the rejection is founded on the alleged fact that the claims cover plant cells expressing an immunoglobulin heavy chain and light chain.

However, as already discussed, the claims are directed to cells that contain nucleic acid encoding an immunoglobulin heavy chain polypeptide but do not contain nucleic acid encoding the light chain. The claims, therefore, do not encompass cells that express a heavy and light chain heterodimer.

It is noted that in the Office Action at page 10 under the section 103 rejection over During, the examiner argues that Goodman teaches to express immunoglobulin heavy and light chains separately as single polypeptides in plants. It is unclear why this argument was present in the rejection over During and was not included in the section 102 (and section 103) rejections over Goodman. In any event, Applicant respectfully submits that there is nothing in Goodman to support this argument. The section in Goodman cited by the examiner to support separate heavy and light chain expression is as follows:

> Structural genes of interest include α -, β - and γ - interferons, **immunoglobulins, with the structural genes coding for the light and heavy chains and desirably assembly occurring in the plant cell**, lymphokines, such as interleukins 1, 2 and 3, growth factors, including insulin-like growth factor, epidermal growth factor, platelet derived growth factor, transforming growth factor-α - β, etc., growth hormone, insulin, collagen plasminogen activator, blood factors, such as factors I to XII, histocompatibility antigens, enzymes, or other mammalian proteins, particularly human proteins

The text in this passage dealing with immunoglobulin has been highlighted in bold for emphasis. The first part, "immunoglobulins, with the structural genes coding for the light and heavy chains" is a reference only to a classic heavy and light chain containing immunoglobulin. The second part, "and desirably assembly occurring in the plant cell" is not a reference to a type of immunoglobulin. Rather, it is a reference to a mode of assembly for the earlier mentioned heavy and light chain immunoglobulin, i.e "in the plant cell" (as opposed to assembly in vitro). Thus, there is nothing in the above cited text or for that matter anywhere else in Goodman that teaches to express immunoglobulin heavy and light chains separately in plants.

Thus, Goodman as a single reference fails to disclose the essential requirement of a plant cell that contains nucleic acid encoding a heavy chain but not a light chain. Anticipation over prior art under 35 U.S.C. § 102 requires the disclosure in a single prior art reference of each element of the claim under consideration. *In re Spada* F.2d, 15 USPQ2d 1655 (Fed. Cir. 1990); *In re Bond*, F.2d, 15 USPQ 1566 (Fed. Cir. 1990); *Soundscriber Corp. v. U.S.* 360 F.2d 954, 148 USPQ 298, 301, *adopted* 149 USPQ 640 (Ct. Cl.) 1966. Accordingly, the rejection for anticipation over Goodman fails and should be withdrawn.

REJECTION UNDER 35 U.S.C. § 103 OVER DÜRING

The rejection of claims 21, 32-54, 56-66 and 68-82 under 35 U.S.C. § 103(a) as being allegedly unpatentable over Düring in view of "Applicant's allegedly admitted prior art for the reasons of record set forth in the office action mailed July 5, 2001" is respectively traversed.

Relevant Law

A claimed invention is obvious if the differences between it and the prior art "are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art." 35 U.S.C. § 103 (1994); see also Graham v. J hn Deere, 383 U.S. 1, 13 (1966). Federal Circuit case law provides that "[t]h consistent criterion for determination f obviousness is whether the prior art

w uld have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in the light of the prior art." In re Dow Chem., 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed.Cir.1988). Under the law, there must be a showing of a suggestion, teaching, or motivation to combine the prior art references is an "essential evidentiary component of an obviousness holding." C.R. Bard, Inc. v. M3 Sys. Inc., 157 F.3d 1340, 1352, 48 USPQ2d 1225, 1232 (Fed.Cir.1998). Also required is that the combined teachings have a reasonable expectation of success, viewed in light of the prior art. *See* In re Dow Chemical Co., 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed.Cir.1988)("Both the suggestion and the expectation of success must be founded in the prior art, not in applicant's disclosure.").

The examiner bears the burden of establishing a *prima facie* case of obviousness. In re Rijckaert, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed.Cir.1993); In re Oetiker, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed.Cir.1992). This showing must be clear and particular, and broad conclusory statements about the teaching of multiple references, standing alone, are not "evidence." *See* Dembiczak, 175 F.3d at 1000, 50 USPQ2d at 1617. However, the suggestion to combine need not be express and "may come from the prior art, as filtered through the knowledge of one skilled in the art." Motorola, Inc. v. Interdigital Technology Corp., 121 F.3d 1461, 1472, 43 USPQ2d 1481, 1489 (Fed.Cir.1997). Only when the examiner's burden is met does the burden of coming forward with rebuttal argument or evidence shift to applicant. Rijckaert, 9 F.3d at 1532, 28 USPQ2d at 1956.

Argument

At the outset, Applicant wishes to address the assertion that the rejection over During is based "Applicant's allegedly admitted prior art for the reasons of record set forth in the office action mailed July 5, 2001." First, it is unclear what is being referred to as "Applicant's allegedly admitted prior art." Applicant is not aware of any admitted prior art and requests that the examiner specify any such art and indicate if and how it is being relied upon in any subs quent rejection.

Sec nd, although the rejection states that it is based on "reasons of record set forth in the office action mailed July 5, 2001," there is no such section 103 rejection over During in that Office Action. The instant rejection, however, states as a basis for the rejection that the claims are not limited to plant cells that contain nucleic acid encoding a heavy chain but not a light chain. The instant rejection also includes a second argument (see top of page 9) that is actually directed to Goodman rather than to During. This apparently misplaced argument has been addressed above in the rebuttal to the section 102 rejection over Goodman. It is therefore concluded that the obviousness rejection over During rests solely on a single argument, that the claims cover plant cells that express an immunoglobulin heavy and light chain heterodimer.

1) **A prima facie case of obviousness is lacking as rejection over During fails to teach or suggest all the elements of the claims**

Thus, the only argument to support the section 103 rejection over During that can be discerned in the instant Office Action, that the claims read on cells that express a heavy and light chain heterodimer immunoglobulin. It is noted that the claims are directed to a plant comprising plant cells containing nucleotide sequence encoding an immunoglobulin heavy chain polypeptide but the cells do not contain nucleic acid encoding the light chain. However, the rejection over During fails to indicate where this reference teaches to use plants to express the heavy chain of immunoglobulin without the light chain. During teaches to express a heterodimeric immunoglobulin and even attempted to express the light chain alone without the heavy chain but the reverse (a heavy chain without a light chain) was never attempted or even mentioned by During.

Furthermore, the instant obviousness rejection over During is defective in failing to cite to any another reference that in combination with During would suggest to express an immunoglobulin heavy chain without the light chain in plants. Applicant respectfully submits that it is the Examiner's initial burden to establish a prima facie case of obviousness. See, e.g., In re Sang-Su Lee, 277 F.3d 1338, 1343 (2002) ([t]he factual inquiry whether to combine references of record must be thorough and searching. It must be based upon objective vidence of r cord.... [P]articular findings must be made as to the

reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed) (internal citations omitted). The Examiner has not met this burden.

It is respectfully submitted, therefore, that the obviousness rejection over During is defective for failing to teach all the elements of the claims. Accordingly, the rejection fails and should be withdrawn.

2) A prima facie case of obviousness over During would be lacking even if other art were cited because motivation to combine and reasonable expectation of success are lacking

Even assuming that the examiner were able to cite to other art to make up for the deficiency in the teachings of During as discussed above, the examiner would also need to show a suggestion, motivation, or teaching in the prior art whereby the person of ordinary skill would have selected the combination of teachings to make the claimed invention and that there would be a reasonable expectation that such combination would be successful. See *Brown & Williamson Tobacco Corp. v. Philip Morris Inc.*, 229 F.3d 1120, 1124-25 (Fed. Cir. 2000).

It is respectfully submitted that any motivation to express the heavy chain without the light chain would derive from the desire to obtain the immunoglobulin heterodimer in plants. However, as extensively discussed by Applicant in a previous response supported by the Lerner declaration, During failed to convincingly demonstrate immunoglobulin heavy and light chain assembly in plants. See declaration and accompanying Amendment filed March 15, 2002. Lerner concludes that a person skilled in the art of immunology or protein expression, circa 1988/1989, would not have reasonably believed the assertion of the During dissertation that plant cells could be used to process and assemble an antigen-specific immunoglobulin. Lerner declaration, ¶ 22. Lerner bases this opinion on During's failure to perform critical controls to support his conclusions and to explain his inconsistent results. Also, the Ac38 antibody which underlies virtually all of the support for During's assertion cannot be used, according to Lerner, to prove that NP antigen specific binding was present in plant cells. Lerner declarati n, ¶ 22. Thus, even if During

had done the proper antigen inhibition controls, much more would have been needed, according to Lerner, to overcome the prejudice in the art. *Id.*

There can be no doubt that Dr. Lerner rejects the notion that During's work would have been considered successful. During cannot be used to support an obviousness rejection when all that During attempts to teach (a fully assembled heavy and light chain immunoglobulin) is not convincing to one of ordinary skill. Lerner has negated this basis in During, leaving nothing upon which to base an obviousness rejection.

Moreover, even if there were motivation to combine During with some other art, there also would be no basis to support the requirement for a reasonable expectation of success. The rejection over During does not even address this requirement. Furthermore, there are several reasons for why it would not have been reasonably expected to successfully express a single immunoglobulin polypeptide of a mutimeric protein by heterologous expression in plants. For example, it was known that polypeptide processing and assembly of a heteromultimer requires support by other proteins in a cell, known as molecular chaparones. For example the Lerner declaration paragraph 4 discusses the involvement of an additional protein called binding immunoglobulin protein or "BiP" in assembly of immunoglobulin. According to Lerner, BiP is located in the endoplasmic reticulum of B cells and binds to heavy chain produced in pre-B cells. In young B cells, heavy chain also binds to BiP, but in the presence of light chain, the heavy chain is released from BiP and the two chains form a fully assembled antibody which is then exported to the cell membrane.[1] Thus, a reasonable expectation of success is lacking because plants are so much different from mammalian cells and it had not been demonstrated as of the 1988/1989 time frame that plant cells even contained a BiP protein or an analogue that would function equivalently.[2]

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[1] See e.g., Bole et al., J. Cell Biology, 102:1558-1566, 1986, APPENDIX 3; Hass et al., Nature 306:387-389, 1983, APPENDIX 4. Lerner Declaration, paragraph 7)

[2] See Mierynk et al., J. Cell Biol., 197: abstract No. 4333, 1988, APPENDIX 13 (titled "Is there a BiP-like protein in the endoplasmic reticulum of plant cells?").

Thus, th known involvement of host proteins in processing the immunoglobulin chains and the absence of knowledge that such host proteins were pres nt in plants supports the absence of a reasonable expectation of success.

Further supporting the absence of a reasonable expectation of success is the phenomenon of heavy chain toxicity. As described by Lerner, unlike the case in pre-B cells, heavy chain production in the absence of light chain production was often fatal in mature B lymphocytes, a phenomenon known as heavy chain toxicity.[3] Heavy chain toxicity was recognized by the absence of myeloma cell mutants that produced heavy but not light chains. Prior to October 1989, is was not clear why heavy chains were toxic to B cells.

The examiner has disregarded the view that heavy chain toxicity is relevant to predictability by citing to Dorai et al. for the proposition that heavy chain toxicity was not a "universal phenomenon of all cell types." There is no requirement that a phenomena be universal for it to raise doubt in the mind of the ordinary skilled artisan. Were that to be the case, then the standard would approach an "absolute" expectation rather than a "reasonable" expectation. Furthermore, Dorai et al., only failed to observe toxicity in a single cell line (SP20 cells). Dorai et al. did not make any predication as to plant cells.

What is important is that heavy chain toxicity was a real phenomenon and was described by several individuals including that of Hass et al. (Appendix 5 to the Lerner Declaration), Morrison et al. (ref. 7 to Dorai et all) and Georges Kohler (Proc. Natl. Acad. Sci. 77:2197-2199, 1980, see abstract), attached herewith. Dorai et al's results with a single mammalian cell would not have eliminated the concept of heavy chain toxicity from the art. Georges Kohler who described heavy chain toxicity is a Nobel laureate in immunology (1984).

It is respectfully submitted that the ordinary skilled artisan would have considered heavy chain toxicity a potential problem in heterologous expression of the heavy chain by itself and would not have reasonably believed that such chains could be successfully expressed in plants with our without the light chain being present.

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[3] See e.g., Hass et al., Proc. Natl. Acad. Sci, USA, 81:7185-7188, 1984, APPENDIX 5, (p.7185, left column).

3) A prima facie case of bviousness ver During w uld be lacking in any case because During teaches away from the claimed invention

As discussed, although During never described or attempted to express the heavy chain in plants without the light chain, During did attempt to express a light chain in the absence of a heavy chain. Lerner Declaration, paragraph 13. As discussed by Lerner, During failed do detect any expression of the light chain alone and did not explain why he failed. Nevertheless, this failure constitutes a teaching away with respect expressing a single immunoglobulin light or heavy chain in plants. The examiner attempts to disregard this view by arguing as follows (see 103 rejection over Goodman, Office Action, page 10):

> Furthermore, During's failure to detect light chain expression would not negate the teachings of Goodman, as the plants taught by During necessarily contain both light and heavy chain immunoglobulin products, as the B1-8 antibody was detected in the plants taught by During (page 112).

This statement is incorrect and suggests that examiner is confusing different experiments. During first attempted to express a light chain in plant cells that did not contain nucleic acid encoding a heavy chain. Lerner Declaration, paragraph 13. During later transfected cells with nucleic acid encoding both a light and a heavy chain. See Lerner declaration beginning at paragraph 14.

4) A prima facie case of obviousness over During would be lacking because During fails to teach the requirement that heavy chain product be capable of forming an antigen-specific immunoglobulin when co-expressed in a plant cell with the light chain from an antigen-specific immunoglobulin.

The claims require that the heavy chain product be capable of forming an antigen-specific immunoglobulin when co-expressed in a plant cell with the light chain from an antigen-specific immunoglobulin. This requirement goes to whether the plant cell which produces the heavy chain is capable of processing the chain such that it would be able to form an antigen specific immunoglobulin were the plant cell to also express th light

chain. During who never successfully expressed a heterodimer immunoglobulin does not teach or suggest this feature of the claims.

The examiner has disregarded this limitation arguing that the ability of a heavy chain to associate with a light chain is an inherent feature of the heavy chain (see section 103 rejection over Goodman, Office Action, page 7). This is not correct. As discussed by Lerner, proper processing and assembly of a single polypeptide via heterologous expression depends on the presence of appropriate chaperone proteins, in particular, the BIP protein as in the case of immunoglobulin. Lerner Declaration, paragraphs 4 and 5. Inherency cannot be present if the existence of critical host proteins plays a role in the biology of proper heavy chain processing. These host proteins may be inherent to the plant cell but that is different from the examiner's position that the processing is inherent to the heavy chain.

Thus the ability of plant cells to support expression of a heavy chain by itself and in a manner that allows the heavy chain to assemble with a light chain in a plant cell was not known nor was it reasonably predicted by During.

5) The obviousness over During is in conflict with the Patent Office's position on restriction

The examiner's position that the various forms of immunoglobulin are nonobvious for purposes of restriction provides another reason to withdraw the obviousness rejection over During. Thus, these arguments manifestly demonstrate that the Patent Office considers the various forms of immunoglobulin as nonobvious variants. Consistency of position requires that the various forms of immunoglobulin be considered non obvious variants for patentability purposes. See also MPEP § 804,01 (Requiring a finding of non-obviousness for claims in a divisional application rejected over the parent application that was subject to restriction).

It is respectfully submitted that the obviousness rejection over During is without basis for any one or more of the above reasons. Accordingly, the rejection fails and should b withdrawn.

REJECTION UNDER 35 U.S.C. § 103 OVER GOODMAN

The rejection of claims 21, 32-54, 56-66 and 68-82 under 35 U.S.C. § 103(a) as being allegedly unpatentable over Goodman for the reasons set forth in the Office Action mailed July 5, 2001, is respectively traversed.

Argument

At the outset, Applicant wishes to address the assertion that the rejection over Goodman is based "Applicant's admitted prior art for the reasons of record set forth in the office action mailed July 5, 2001." It is unclear what is being referred to as "Applicant's admitted prior art." Applicant is not aware of any admitted prior art and requests that the examiner specify any such art and indicate if and how it is being relied upon in any subsequent rejection.

The rejection states that it is based on reasons of record in the Office Action mailed July 5, 2001. That Office Action contains a section 103 rejection over Goodman but refers to an earlier Office Action for the grounds of support. The Office Action mailed October 12, 2000 appears to be the Office Action that contains the examiner's earlier reasoning.

As best as Applicant can determine, the section 103 rejection over Goodman in the Office Action mailed October 12, 2000 asserts that Goodman teaches to express a heavy and light chain containing antibody in plants. The examiner also argued in the instant Office Action that the claims cover cells expressing a heavy and a light chain. In addition, the instant Office Action asserts on page 10 what appears as a new ground for rejection over Goodman.

> The office further maintains that Goodman's expression of a heterologous interferon polypeptide in plant cells would reasonably have been considered to advance the possibility of expressing any single chain heterologous polypeptide in a plant cell. While gamma interferon is structurally and functionally distinct from immunoglobulins, b th gamma interfer n and immunoglobulins are heter logous to plants.

Applicant rebuts both of these arguments below.

1) A prima facie case of obviousness is lacking as rejection fails to teach or suggest all the elements of the claims

The claims are directed to cells a plant comprising plant cells containing nucleotide sequence encoding an immunoglobulin heavy chain polypeptide but the cells do not contain nucleic acid encoding the light chain. However, the rejection over Goodman fails to indicate where this reference teaches to use plants to express the heavy chain of immunoglobulin without the light chain. Goodman suggested that plants could be used to express a variety of heterologous proteins. Goodman lists a variety of proteins including many that are naturally expressed as a single polypeptide (e.g., interleukins 1, 2 and 3) and a few which are heteromultimeric including immunoglobulin, fibrinogen and histocompatiblity antigens (fibrinogen comprises an Aα (610 residues), a Bβ (461 residues) and a gγ (411 residues) chain; class I MHC antigen comprises a heavy chain and beta-2 microglobulin; class II comprises an α chain and a β chain).

Apparently aware of this problem, the examiner now argues that Goodman actually teaches to express the heavy chain and the light chain separately (this new argument was misplaced in the section 103 rejection over During, Office Action, page 9). As already addressed above under the rebuttal to the section 102 rejection over Goodman, there is nothing in Goodman that teaches to separately express any individual polypeptide of a heterologous heteromeric protein.

Furthermore, the instant obviousness rejection over Goodman is defective in failing to cite to any another reference that in combination with Goodman would suggest to express an immunoglobulin heavy chain without the light chain in plants. Applicant respectfully submits that it is the Examiner's initial burden to establish a prima facie case of obviousness. See, e.g., In re Sang-Su Lee, 277 F.3d 1338, 1343 (2002) ([t]he factual inquiry whether to combine references of record must be thorough and searching. It must be based upon objective evidence of record.... [P]articular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these comp nents for combination in the manner claimed) (internal citations mitted). The Examiner has not met this burden.

It is respectfully submitted, therefore, that the obviousn ss rejection over Goodman is defective for failing to teach all the elements of the claims. Accordingly, the rejection fails and should be withdrawn.

2) A prima facie case of obviousness over Goodman would be lacking even if other art were cited because motivation to combine and reasonable expectation of success are lacking

Even assuming that the examiner were able to cite to other art to make up for the deficiency in the teachings of Goodman, the examiner would also need to show a suggestion, motivation, or teaching in the prior art whereby the person of ordinary skill would have selected the combination of teachings to make the claimed invention and that there would be a reasonable expectation that such combination would be successful. See *Brown & Williamson Tobacco Corp. v. Philip Morris Inc.*, 229 F.3d 1120, 1124-25 (Fed. Cir. 2000).

It is respectfully submitted that any motivation to express the heavy chain without the light chain would derive from the desire to obtain the immunoglobulin heterodimer in plants. However, as extensively discussed herein and supported by the declaration of Richard Lerner, there was a prejudice in the art against such immunoglobulin heterodimer expression in plants. It has also has been demonstrated by Lerner that the During dissertation evidences an attempt to obtain such heterodimer expression of functional immunoglobulin heterodimer but because of its many deficiencies, fails to overcome the prejudice. Thus, there would be no motivation to avail a rejection over a combination of art that included Goodman, even if the examiner were to find such art.

Moreover, even if there were motivation to combine, there also would be no basis to support the requirement for a reasonable expectation of success. Relevant to the issue is the statement by the examiner in the rejection over Goodman that "gamma interferon and immunoglobulins are heterologous to plants." However, this fails to support a reasonable expectation of success. Being "heterologous" is not a matter of similarity or dissimilarity. Rather, it is only a r ference to expression in a foreign host cell; one that is a different phyogenetic class from a cell that naturally expresses the protein. A protein

from E. *coli* is h terol g us to S. *aueus* while a protein from human is also heter logous to S. *aureus*. The fact that the E. *coli* protein and the human protein are both heterologous to S. *aureus* has absolutely nothing to do with whether success with expressing one of these two proteins is reasonably predictive of the other. It is respectfully submitted that the examiner's reasoning that both interferon and immunoglobulin are heterologous offers nothing to rebut the admitted fact that these two proteins are structurally and functionally distinct, a fact which argues against predictability of expression in plants where Goodman supported by another necessary teaching.

Furthermore, there are several reasons for why successfully expressing a naturally single polypeptide would not be considered to reasonably predict success for expressing a single chain of a mutimeric protein. For example, it was known that polypeptide processing and assembly of a heteromultimer requires support by other proteins in a cell, known as molecular chaparones. For example the Lerner declaration paragraph 4 discusses the involvement of an additional protein called binding immunoglobulin protein or "BiP" in assembly of immunoglobulin. According to Lerner, BiP is located in the endoplasmic reticulum of B cells and binds to heavy chain produced in pre-B cells. In young B cells, heavy chain also binds to BiP, but in the presence of light chain, the heavy chain is released from BiP and the two chains form a fully assembled antibody which is then exported to the cell membrane.[4] Importantly, it had not been demonstrated as of the 1988/1989 time frame that plant cells even contained a BiP protein or an analogue that would function equivalently.[5]

Thus, the known involvement of host proteins in processing the immunoglobulin chains and the absence of knowledge that such host proteins were present in plants supports the absence of a reasonable expectation of success.

Further supporting the absence of a reasonable expectation of success is the phenomenon of heavy chain toxicity. As described by Lerner, unlike the case in pre-B

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[4] See e.g., Bole et al., J. Cell Biology, 102:1558-1566, 1986, APPENDIX 3; Hass et al., Nature 306:387-389, 1983, APPENDIX 4.

[5] See Mierynk et al., J. Cell Biol., 197: abstract No. 4333, 1988, APPENDIX 13 (titled "Is there a BiP-like protein in the endoplasmic reticulum of plant cells?").

cells, heavy chain production in the absence of light chain production was often fatal in mature B lymphocytes, a phenomenon known as heavy chain toxicity.[6] Heavy chain toxicity was recognized by the absence of myeloma cell mutants that produced heavy but not light chains. Prior to October 1989, is was not clear why heavy chains were toxic to B cells.

The examiner has disregarded the view that heavy chain toxicity is relevant to predictability by citing to Dorai et al. for the proposition that heavy chain toxicity was not a "universal phenomenon of all cell types." There is no requirement that a phenomena be universal for it to raise doubt in the mind of the ordinary skilled artisan. Were that to be the case, then the standard would approach an "absolute" expectation rather than a "reasonable" expectation. Furthermore, Dorai et al., only failed to observe toxicity in a single cell line (SP20 cells). Dorai et al. did not make any predication as to plant cells.

What is important is that heavy chain toxicity was a real phenomenon and was described by several individuals including that of Hass et al. (Appendix 5 to the Lerner Declaration), Morrison et al. (ref. 7 to Dorai et all) and Georges Kohler (Proc. Natl. Acad. Sci. 77:2197-2199, 1980, see abstract), attached herewith. Dorai et al's results with a single mammalian cell would not have eliminated the concept of heavy chain toxicity from the art. Georges Kohler who described heavy chain toxicity is a Nobel laureate in immunology (1984).

It is respectfully submitted that the ordinary skilled artisan would have considered heavy chain toxicity a potential problem in heterologous expression of the heavy chain by itself and would not have reasonably believed that such chains could be successfully expressed in plants with our without the light chain being present.

3) A prima facie case of obviousness over Goodman would be lacking

In any case because During teaches away from the claimed invention

As discussed, although During never described or attempted to express the heavy chain in plants without the light chain, During did attempt to express a light chain in the absence of a heavy chain. Lerner Declaration, paragraph 13. As discussed by Lerner,

[6] See e.g., Hass et al., Proc. Natl. Acad. Sci, USA, 81:7185-7188, 1984, APPENDIX 5, (p.7185, left column).

During fail d d d tect any expression of the light chain al ne and did not explain why he failed. Nevertheless, this failure constitutes a teaching away with respect expr ssing a single immunoglobulin light or heavy chain in plants. The examiner attempts to disregard this view by arguing as follows (see 103 rejection over Goodman, Office Action, page 10):

> Furthermore, During failure to detect light chain expression would not negate the teachings of Goodman, as the plants taught by During necessarily contain both light and heavy chain immunoglobulin products, as the B1-8 antibody was detected in the plants taught by During (page 112).

This statement is incorrect and suggests that examiner is confusing different experiments. During first attempted to express a light chain in plant cells that did not contain nucleic acid encoding a heavy chain. Lerner Declaration, paragraph 13. During later transfected cells with nucleic acid encoding both a light and a heavy chain. See Lerner declaration beginning at paragraph 14.

4) **A prima facie case of obviousness over Goodman would be lacking because Goodman fails to teach the requirement that heavy chain product be capable of forming an antigen-specific immunoglobulin when co-expressed in a plant cell with the light chain from an antigen-specific immunoglobulin.**

The claims require that the heavy chain product be capable of forming an antigen-specific immunoglobulin when co-expressed in a plant cell with the light chain from an antigen-specific immunoglobulin. This requirement goes to whether the plant cell which produces the heavy chain is capable of processing the chain such that it would be able to form an antigen specific immunoglobulin were the plant cell to also express the light chain. Goodman who never successfully expressed a heterodimer immunoglobulin does not teach or suggest this feature of the claims.

The examiner has disregarded this limitation arguing that the ability of a heavy chain to associate with a light chain is an inherent feature of the heavy chain (see Office Action, page 7). This is not correct. As discuss d by Lerner, proper processing and assembly f a single polypeptide via heterolog us expression depends on the presence of

appropriate chaperone prot ins, in particular, the BIP protein as in the case of immunoglobulin. Lerner Declaration, paragraphs 4 and 5. Inherency cannot be present if the existence of critical host proteins plays a role in the biology of proper heavy chain processing. These host proteins may be inherent to the plant cell but that is different from the examiner's position that the processing is inherent to the heavy chain.

Thus the ability of plant cells to support expression of a heavy chain by itself and in a manner that allows the heavy chain to assemble with a light chain in a plant cell was not known nor was it reasonably predicted by Goodman.

5) The obviousness over Goodman is in conflict with the Patent Office's position on restriction

The examiner's position that the various forms of immunoglobulin are nonobvious for purposes of restriction provides another reason to withdraw the obviousness rejection over During. Thus, these arguments manifestly demonstrate that the Patent Office considers the various forms of immunoglobulin as nonobvious variants. Consistency of position requires that the various forms of immunoglobulin be considered non obvious variants for patentability purposes. See also MPEP § 804,01 (Requiring a finding of non-obviousness for claims in a divisional application rejected over the parent application that was subject to restriction).

It is respectfully submitted that the obviousness rejection over Goodman is without basis for any one or more of the above reasons. Accordingly, the rejection fails and should be withdrawn.

Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested. The Examiner is urged to contact the undersigned by telephone to address any outstanding issues standing in the way of an allowance.

Atty. Dkt. No. EPI3007B
(formerly TSRI 184.2C2)

Respectfully submitt d,

By _____

Barry S. Wilson
Attorney for Applicant
Registration No. 39,431

Date: April 2, 2003

FOLEY & LARDNER
P.O. Box 80278
San Diego, California 92138-0278
Telephone: (858) 847-6700
Facsimile: (858) 792-6773

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Immunology

Immunoglobulin chain loss in hybridoma lines

(immunoglobulin variants/chromosome loss)

GEORGES KÖHLER

Basel Institute for Immunology, 487 Grenzacherstrasse, Postfach, 4005 Basel, Switzerland

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ABSTRACT Hybrid cells secreting one, two, or three different immunoglobulins were constructed. The loss of immunoglobulin heavy or light chain expression was monitored. Chain loss was random only in lines with an excess of active light chain genes over heavy chain genes. In all other combinations preferential heavy chain loss was observed. Variant cells altered in heavy or light chain synthesis exhibited an altered chain loss pattern. It is therefore proposed that free immunoglobulin heavy chain is toxic for the cells. The interdependence of the two gene products gives a possible molecular explanation of apparent directed chromosome loss in hybrid cells.

If a specialized cell, such as a B lymphocyte, produces a large amount of a protein that, like immunoglobulin (Ig), is composed of two different polypeptide chains, one or the other chain will, in general, be synthesized in excess. That is, precise stoichiometry is a special case, which would require a special mechanism to achieve and which is, indeed, not achieved in most cases. B lymphocytes, for example, produce an excess of light (L) chains over heavy (H) chains. Natural selection, during ontogeny as well as during phylogeny, will ensure that the free form of the chain made in excess will not be toxic for the cell synthesizing it. But there will be little or no comparable selective pressure against the free form of the one not produced in excess. Therefore, we would expect that experimentally reversing the ratio of chain syntheses would prove to be detrimental or even lethal to the cell.

The Ig system of the mouse is particularly suitable for studying this possibility. Ig is secreted in large quantities by myeloma and hybridoma lines. The H and L chains of different Igs can easily be discriminated on $NaDodSO_4$/polyacrylamide gels. In Ig-secreting mouse–mouse hybridoma cells (similar to those used in this study) H or L chain loss was correlated with the loss of one copy of chromosome 12 or 6, respectively. Only one of the two homologous chromosomes directs Ig synthesis (1). Hence, chain loss due to chromosome loss will be an all or none phenomenon easy to detect. Early studies on myeloma cells indicated that loss of H chain expression was a frequent event that preceded the loss of L chain expression (2). This observation is compatible with the idea that free H chain is toxic for the cell. The present study reinforces this notion.

MATERIAL AND METHODS

Cell Lines and Culture Conditions. Cells were grown in Dulbecco's modified Eagle's medium (GIBCO) supplemented to contain penicillin and streptomycin at 100 units/ml each, 15% heat-inactivated fetal bovine serum, and 50 μM mercaptoethanol. The lines used in this study are summarized in Table 1.

Cell Fusions. Hybrids expressing two H and three L chains were obtained by fusing Sp1/HL-Ag (Table 1) and Sp2B-BU [the μ chains differ in size (8); the L chains can be discriminated by using isoelectric focusing analysis]. Hybrids secreting three H and three L chains were obtained by fusing P1BU1-Ou with Sp2/HLML′ and Sp2/HL-BU with Sp25/5-1 Ag13 (in both fusions all chains differ in $NaDodSO_4$/polyacrylamide gel electrophoresis). Fusions were performed with 3×10^6 cells of each of the parental lines in the presence of 0.7 ml of 50% (vol/vol) polyethylene glycol 1500 (British Drug House, England) in serum-free Dulbecco's modified Eagle's medium. Cells were divided into 24 1-ml cultures. In most cases cultures with hybrids were cloned before being analyzed for Ig chain loss. This was omitted when fewer than 10 hybrids grew out of 24 initial cultures.

Table 1. Cell lines

Code	Line	Ig secretion	Origin	Ref.
A	X63-Ag8	γ_1, κ	BALB/c myeloma	3
B	P1BU1-Ou	γ_{2a}, κ	BALB/c myeloma	3
C	Sp1/HL-Ag	μ, κ	Hybrid of A	4
D	Sp2/HL-BU	γ_{2b}, κ	Hybrid of A	4
E	Sp2/0-Ag14	None	Hybrid of A	5
F	Sp25/5-1-Ag13	$(\mu, \kappa) + (\gamma_1, \kappa)$	Hybrid of A	6
G	Sp6/HLGK	$(\mu, \kappa) + (\gamma_1, \kappa)$	Hybrid of A	4
H	Sp2/01-Ag	None	Hybrid of E	
K	Sp2/HLML′-Ag	$(\mu, L) + (\gamma_{2b}, \kappa)$	Hybrid of D	7
L	Sp2B-BU	$(\mu, L) + (\kappa)$	Hybrid of D	8

Ag, BU, and Ou stand for resistance to 8-azaguanine at 20 μg/ml, 5-bromo-2′-deoxyuridine at 30 μg/ml, and 5 mM ouabain, respectively.

Analysis of Chain Loss. Soft agar cloning, radioactivity incorporation, $NaDodSO_4$/polyacrylamide gel electrophoresis, and isoelectric focusing using reduced radiolabeled culture supernatants were performed as described (4, 8).

RESULTS

The chain loss of many different hybrids is summarized in this section. Because many of the original hybrids were obtained by using the X63-Ag8 line, care was taken to include hybrids made by other lines (Sp2/HL-BU; P1BU1-Ou) as well. No difference was observed in their chain loss pattern.

Expression of Immunoglobulin H and L Chains. Loss of Ig-chain expression of randomly picked clones was measured by analyzing labeled and reduced culture supernatants on $NaDodSO_4$/polyacrylamide gel electrophoresis. Fig. 1 shows the pattern obtained from the (D–F) hybrid (see Table 1), which

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Abbreviations: H chain, heavy chain; L chain, light chain.

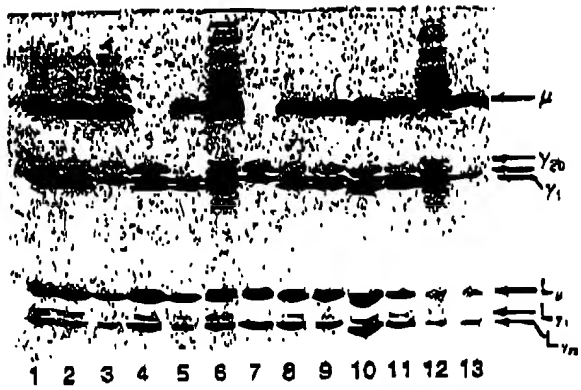

FIG. 1. Hybrid D–F expressing three different Igs. [^{14}C]Leucine-labeled culture supernatants of 13 clones were analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis under reducing conditions. The H chain suffixes given to the L chains indicate their original association.

secretes three different Igs. It can be observed that 2 out of 13 clones lost the secretion of the μ chain and that 3 clones lost the secretion of the γ_1 chain.

These losses are included in group I of Table 2. In this group, for example, two different hybrids (lines) expressing three immunoglobulins were characterized. Ten clones and 182 isolates of these hybrids were analyzed as 33 H chain and 9 L chain losses (observed values). From these, 12 H and 7 L losses were independently obtained. This means that multiple losses of the same H and L chain detected in the isolates of one clone will score as only one independent loss of this particular chain. This avoids the problem of repeats, which may obscure the results (see the L chain losses in group VI: six of the eight observed losses came from one clone). The expected value is based on the independent numbers, which in the example of group I is 9.5 each for H and L, assuming random chain loss.

Chain loss was random among L chains (mostly κ class) and among H chains, irrespective of H chain class, but not when L or H chain losses were compared to each other. Analysis of many different hybrid lines (Table 2) shows that H and L chains are lost randomly only in combinations in which there is a greater number of active genes for L chains than for H chains, except for the highest combination (3H + 3L). All other combinations that expressed a number of L chains equal to or less than the number of H chains showed preferential H chain loss.

Table 2. Summary of Ig chain losses

Group	Lines/ clones/ isolates	Chain combination	Observed/independent and (expected) loss of H	Observed/independent and (expected) loss of L
I	2/10/182	3H 3L	33/12 (9.5)	9/7 (9.6)
II	2/4/70	2H 3L	5/3 (3.6)	17/6 (5.4)
III	1/1/11	1H 3L	1/1	1/1
IV	1/2/26	0H 3L	—	2/2
V	2/5/98	3H 2L	15/8 (4.8)	0/0 (3.2)
VI	12/17/647	2H 2L	46/24 (13.5)	8/3 (13.5)
VII	6/6/446	1H 2L	5/5 (4.3)	11/8 (8.7)
VIII	8/9/116	1H 1L	9/7 (3.5)	0/0 (3.5)

Group I, hybrids between D–F and B–K (for code of letters see Table 1); group II, D–F and C–L; group III, D–F; group IV, C–L; group V, as I; group VI includes groups I, II, and A and D times mouse lymphocytes; group VII, A times mouse lymphocytes; group VIII, E and H times mouse lymphocytes. The expected number is based on the independent losses (see text) and gives the values of random loss of H and L chain expression. The boxed chain combinations gave values not compatible with random loss of H and L chain expression (χ^2 test, one degree of freedom, probability level 5%).

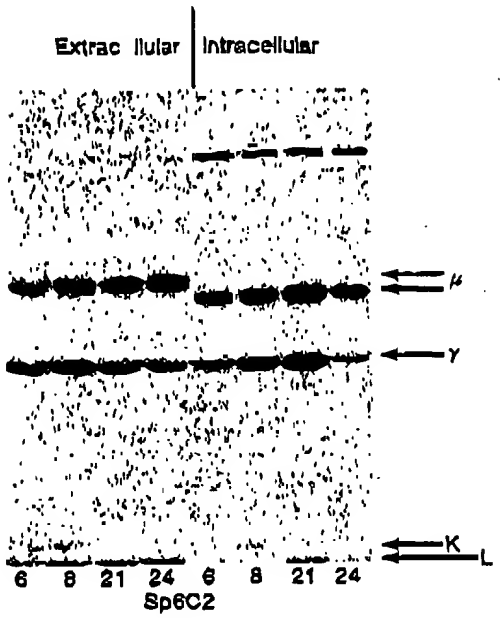

FIG. 2. Differential secretion of K chains in clones of the hybridoma line Sp6C2 (left). Detectable amounts of K were found intracellularly (right) after NaDodSO$_4$/polyacrylamide gel electrophoresis under reducing conditions.

Alteration in H or L Chain Synthesis Changes Chain Loss Pattern. The line Sp6/HLGK secretes, in addition to the myeloma X63-Ag8 γ (G) and κ (K) chains, μ (H) and κ (L) chains with anti-trinitrophenyl specificity (4, 8). Two types of clones exist for this line: those that secrete normal amounts of K (Fig. 2, Sp6C2/6 and 8) and those that secrete very little K (Fig. 2, Sp6C2/21 and 24). Both types show easily detectable intracellular K chains (9). Random clones of all four lines show preferential H chain loss: 14 and 32 out of 125 clones analyzed showed loss of the μ and γ_1 chain, respectively. No light chain loss was observed. In this screening a variant of μ chain was isolated twice out of the 42 clones analyzed from the Sp6C2/6 line. The variant μ of Sp6C2/6-46 was about 10,000 daltons smaller than wild type μ. Its IgM was multimeric and had anti-trinitrophenyl activity but did not have the light chains covalently bound. When 15 subclones of this variant were analyzed for chain loss, 2 had lost K chain production. This behavior was quite unlike that of the parental lines and group VI in Table 2.

Selection for specificity loss in two independent Sp6/HLk clones (lower-case k symbolizing the phenotype of clones secreting only small amounts, similar to Sp6C2/21 and 24 in Fig. 2), showed that H chain loss was about 1000 times more frequent than L chain loss (9). However, when a similar selection was performed on C-loss variants of Sp6C2/6 and 8 (Fig. 2), H and L were lost equally often, following the pattern of group VII in Table 2.

DISCUSSION

Hengartner *et al.* (1) showed directly that mouse H or L chain loss was correlated with the loss of one copy of chromosome 12 or chromosome 6, respectively. Here we have demonstrated that H and L chain losses, which probably result from losses of their corresponding coding chromosomes, are random unless an excess number of H chains over L chains is expressed by the

cell, a condition that seems to be deleterious to the cell. This restriction seems, however, to relax at higher chain combinations (3H and 3L, Table 2). Perhaps the two remaining L chains make enough product to be equivalent to that of the three H chain genes. This would require a 1.5-fold molar excess of L over H chains, a value well in agreement with measurements in myeloma and mouse lymph node cells (10). The restriction becomes prominent at 2H and 2L and is quite severe in 1H + 1L combination, which is the normal myeloma situation, as Coffino and Scharff (2) have analyzed in detail for the MPC-11 line and Cowan *et al.* (11) for the P8 line. In screening several hundred thousand cells they never found a producer of free H chain [except when the H chain was itself modified (11, 12)], although L chain losses occurred after H chain loss at a frequency of 4×10^{-3} per cell per generation (2). Similar observations have now been made with several hybridoma lines expressing only 1H and 1L (group VIII of table 2; ref. 4).

Could the apparent nonrandom chain and chromosome loss be explained by a deleterious gene dosage effect of a product of chromosome 12 other than the free H chain itself? This is unlikely, as indicated by a series of observations. First, the pattern of chain and chromosome losses is best explained by an interdependence of chromosome 12 and 6. The chromosomes not expressing H and L chains, known to be present in uncertain numbers, but at least once (1), seem not to randomize the results. Second, deletions in the region of the first constant H chain domain alter the chain loss pattern (Sp6C2/6-4S cells). From a similar deletion variant of the MPC-11 (IgG_{2b}) line, Morrison (12) isolated a cell line that produces only H chains, and Milstein and coworkers (11, 13) isolated such a line from another deletion variant line, derived from P3 (IgG_1). At least some of these deletions seem to mimic those found in human H chain disease, in which variant H chains are made in the absence of L chains (14). Third, changing the amount of L chain being secreted again seems to change the normal random H + L loss pattern of the group VII type (Table 2; ref. 9). Exceptions to the idea of free H chains being toxic to the cell have been reported (15). It is interesting that pre-B cells seem to make free μ chains without secreting them (16). I would suggest that in these cases variant H chains are produced.

The hypothesis of chromosomes being interrelated by some of their gene products leads to an apparent directed chromosome loss in the hybrids studied. It may well be that other such interdependencies operate, implying that "pathways" of chromosome losses may exist in hybrid cells. This could explain why in one fusion some hybrids are difficult to grow and others are not. The poor growers may have started with an early random chromosome loss that leads, possibly only after several other chromosome losses, to an incompatible chromosomal complement.

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